

Genetic Diversity in Two Island Endemics, *Hosta venusta* and *H. tsushimensis* (Liliaceae)

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Levels of genetic diversity and population structure in *Hosta venusta* and *H. tsushimensis*, herbaceous perennials endemic to Cheju Island and Tsushima Island, respectively, were investigated using allozyme markers. Although the distributions of the species are restricted in the islands, they maintain high levels of genetic variation (mean $H_e = 0.296$ and 0.215 for the populations of *H. venusta* and *H. tsushimensis*, respectively) and the degrees of population differentiation were lower than the species of *Hosta* with similar life history traits and widespread distribution range such as *H. capitata* and *H. clausa*. The findings suggest that *H. venusta* and *H. tsushimensis* may have large effective population sizes.

Recent reviews of plant allozyme literature have shown causal relationships between life history and ecological traits and the amount and patterns of genetic variation (Hamrick and Godt 1989, Hamrick, Godt and Sherman-Broyles 1992). For example, species with endemic distributions maintain lower level of genetic diversity than species with widespread distributions. However, several studies reported that geographic range is not always a good predictor of the genetic structure of species (Karron et al. 1988, Olmstead 1990, Soltis and Soltis 1991, Godt and Hamrick 1993, Chung and Chung 1994). This may be largely explained by two possible explanations. First, species, even congeners, often differ in many other aspects of their biology. Second, relatively fewer allozyme studies of endemic or rare species, particularly island endemic species, are available in the plant allozyme literature.

In this study I investigate levels and distribution of genetic variation in *Hosta venusta* F. Maekawa and *H.*

tsushimensis N. Fujita, herbaceous perennials restricted to Cheju Island, Korea and Tsushima Island, Japan. *Hosta venusta* occurs commonly on grassland and understory of reforestation areas, while *H. tsushimensis* grows on diverse habitats such as forest margin, valleys and rocky areas (Fujita 1976; Chung pers. obs). Like other hostas, these two species are insect-pollinated. Flowers are visited by bees and seeds with wing are dispersed by wind. Chromosome number is $2n = 60$ (Chung et al. 1991a).

Materials and Methods

A total of 124 samples of *H. venusta* and *H. tsushimensis* rootstocks were collected from four localities in Cheju and Tsushima Islands. Table 1 lists the four populations, their alphabetic codes, sample sizes, and vouchers used in this study. Collections of rootstocks from each site were made at least 2 m apart to reduce the likelihood of collecting clones. The rootstocks were transported to the Botany Plant Growth

Table 1. Population code, location of the four populations of *H. venusta* and *H. tsushimensis*

Code	Locality	N ^a
<i>H. venusta</i>		
A	Cheju Island, Cheju city, Korea	32
B	Cheju Island, Samgumburi, Pukcheju-gun, Korea	31
<i>H. tsushimensis</i>		
C	Tsushima Island, Izuhara, Pref., Japan	27
D	Tsushima Island, Mt. Ariake, Japan	34

^aSample size.

Facility at the University of Georgia where plants were grown under uniform conditions for electrophoretic analyses. Voucher specimens of all collections were deposited at GA, GNUC, KYO, MO, and SNU.

Leaf tissue for each sample was crushed in liquid nitrogen with a mortar and pestle, and proteins were extracted using a phosphate polyvinylpyrrolidone buffer (Mitton et al. 1979). Protein extracts were absorbed onto Whatman 3 mm chromatography paper wicks, placed in 96-well microtest plates and stored at -70°C . Electrophoresis was performed using 11% starch gels. Except as noted, gel and electrode buffers and enzyme staining procedures from Soltis et al. (1983) were used to assay ten enzyme systems: isocitrate dehydrogenase (IDH) on system 2; shikimate dehydrogenase (SKDH) on system 4; phosphoglucosmutase (PGM), diaphorase (DIA), and glutamate oxaloacetate transaminase (GOT) on system 7; leucine aminopeptidase (LAP), triosephosphate isomerase (TPI), β -galactosidase (β GAL), and fluorescent esterase (FE) on a modification (Haufler 1985) of system 8. The staining procedures for DIA and β GAL followed the method described by Cheliak and Pitel (1984). Putative loci were designated sequentially, with the most anodally migrating isozyme designated 1, the next 2, and so on. Likewise, alleles were designated sequentially with the most anodally migrating alleles designated a.

Levels of allozyme variation were estimated at the species level and for individual populations with a computer program developed by M. D. Loveless and A. Schnabel. A locus was considered polymorphic if two or more alleles were observed. Genetic diversity was measured by four parameters: percent polymorphic loci (P), average number of alleles per locus (A), mean effect number of alleles per locus (Ae), and gene diversity or expected heterozygosity (He). The statistics of the four parameters were well described in Hamrick and Godt (1989) and Hamrick, Godt, and Sherman-Broyles (1992).

Observed heterozygosity, expected heterozygosity, and Wright's (1922) fixation indices (F) were calculated for each locus and for each population to reveal significant deviations in genotype frequencies from expectations under Hardy-Weinberg equilibrium. Positive values of F indicate an excess of homozygotes and negative values indicate an excess of heterozygotes within the populations. Significance of the F coefficients was tested by a χ^2 -statistic following Li and Horvitz (1953).

Allozyme variation was partitioned within and among populations for each locus using Nei's (1973, 1977) genetic diversity formulae. The total gene diversity for polymorphic loci (H_T) was partitioned into within population (H_S), and among population (D_{ST}) components. The proportion of genetic diversity found among populations was calculated as $G_{ST} = D_{ST}/H_T$. G_{ST} values were tested for significance by $\chi^2 = 2NG_{ST}(a-1)$; $df = (a-1)(n-1)$, where N is the total number of individuals analyzed, a is the number of alleles at the locus and n is the number of populations (Workman and Niswander 1970). Nei's (1972) genetic identities and distances were calculated for pairwise combination of populations and species.

Results

Sixteen and 11 of 24 loci were polymorphic in *H. venusta* and *H. tsushimensis*, respectively. Three alleles

were observed for five loci of the two species, with the total average numbers of alleles for the two species being 1.88 and 1.42, the average effective number of alleles 1.57 and 1.40, and overall genetic diversity 0.315 and 0.208. Populations of *H. venusta* and *H. tsushimensis* maintained high levels of genetic variation: the mean percentage of polymorphic loci of 66 and 45% and the mean genetic diversity of 0.296 and 0.215 (Table 2).

Fifteen (eight positive; seven negative) of 32 fixation indices in *H. venusta* and 11 (seven positive; four negative) in *H. tsushimensis* were significantly different from zero (Table 3). On the whole, allele frequencies were close to Hardy-Weinberg expectations with mean F_{IS} of -0.034 and 0.027 for *H. venusta* and *H. tsushimensis*.

Mean total genetic diversity at the polymorphic loci (H_T) for *H. venusta* (0.473) was very comparable

Table 2. Summary of allozyme variation for 24 loci within populations of *H. venusta* and *H. tsushimensis*: proportion of polymorphic loci (P), mean number of alleles per locus (A), mean effective number of alleles per locus (Ae), mean observed heterozygosity (Ho), and mean expected heterozygosity (He).

Code	P	A	Ae	Ho (SD)	He (SD)
<i>H. venusta</i>					
A	66.67	1.83	1.57	0.272 (0.065)	0.286 (0.048)
B	66.67	1.88	1.58	0.344 (0.064)	0.305 (0.046)
Mean	66.67	1.86	1.57	0.308 (0.046)	0.296 (0.033)
<i>H. tsushimensis</i>					
C	45.83	1.42	1.44	0.193 (0.056)	0.215 (0.049)
D	45.83	1.42	1.43	0.186 (0.051)	0.216 (0.048)
Mean	45.83	1.42	1.44	0.189 (0.038)	0.215 (0.034)

Table 3. Fixation indices (F) for 16 polymorphic loci of *H. venusta* and 11 polymorphic loci of *H. tsushimensis*

Locus	<i>H. venusta</i>		<i>H. tsushimensis</i>	
	A	B	C	D
<i>Idh</i>	-0.4318^{**}	-0.3864^*	-0.5588^{***}	-0.5581^{***}
<i>Skdh-2</i>	-0.1250^{ns}	0.5770^{**}	0.3690^{ns}	-0.1917^{ns}
<i>Pgm-1</i>	-0.0862^{ns}	-0.2449^{ns}	—	—
<i>Pgm-2</i>	-0.2353^{ns}	-0.2000^{ns}	-0.3590^{ns}	-0.5227^{***}
<i>Pgm-3</i>	-0.3404^{ns}	-0.2449^{ns}	0.4692^{**}	0.6036^{***}
<i>Dia-1</i>	0.3848^*	0.1712^{ns}	—	—
<i>Dia-2</i>	-0.5000^{**}	-0.6053^{***}	-0.0192^{ns}	-0.3958^*
<i>Dia-3</i>	-0.1982^{ns}	-0.6138^{ns}	0.7017^{***}	0.4591^{**}
<i>Got-1</i>	—	—	0.1452^{ns}	0.5550^{***}
<i>Got-2</i>	0.5714^{***}	0.5841^{***}	-0.1451^{ns}	0.0337^{ns}
<i>Got-3</i>	0.0762^{ns}	-0.6620^{***}	-0.3701^{ns}	-0.0828^{ns}
<i>Pgd-1</i>	0.4337^{**}	-0.2931^{**}	0.0063^{ns}	0.1434^{ns}
<i>Lap-3</i>	0.5333^{***}	-0.1200^{ns}	—	—
<i>Tpi-2</i>	-0.0021^{ns}	0.6155^{***}	0.4960^{***}	0.6240^{***}
<i>Tpi-3</i>	0.1703^{ns}	-0.2380^{ns}	—	—
<i>Fe-2</i>	0.6921^{***}	0.6013^{***}	—	—
<i>Fe-3</i>	-0.1564^{ns}	-0.5250^{***}	—	—

Table 4. Nei's (1973, 1977) statistics of genetic diversity for 16 polymorphic loci in *H. venusta* and 11 polymorphic loci in *H. tsushimensis*. A χ^2 test for allele frequency heterogeneity between populations is given: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Locus	<i>H. venusta</i>					<i>H. tsushimensis</i>				
	H _T	H _S	D _{ST}	G _{ST}	χ^2	H _T	H _S	D _{ST}	G _{ST}	χ^2
<i>Idh</i>	0.4213	0.4210	0.0002	0.0006	0.074(2) ^{ns}	0.4656	0.4656	0.0000	0.0000	0.001(2) ^{ns}
<i>Skdh-2</i>	0.4274	0.3571	0.0703	0.1645	20.722(1) ^{***}	0.4020	0.3970	0.0051	0.0126	1.537(1) ^{ns}
<i>Pgm-1</i>	0.2561	0.2494	0.0067	0.0262	3.305(1) ^{ns}					
<i>Pgm-2</i>	0.4058	0.3987	0.0071	0.0174	4.863(2) ^{ns}	0.4350	0.4322	0.0028	0.0064	0.782(2) ^{ns}
<i>Pgm-3</i>	0.3628	0.3612	0.0016	0.0043	0.543(3) ^{ns}	0.4192	0.4159	0.0033	0.0079	1.592(3) ^{ns}
<i>Dia-1</i>	0.4717	0.4424	0.0293	0.0620	7.816(1) ^{**}					
<i>Dia-2</i>	0.4636	0.4627	0.0009	0.0020	0.255(1) ^{ns}	0.2956	0.2630	0.0260	0.1103	13.458(1) ^{***}
<i>Dia-3</i>	0.4898	0.4897	0.0001	0.0002	0.024(2) ^{ns}	0.4988	0.3708	0.1280	0.2566	31.299(2) ^{***}
<i>Got-1</i>						0.5575	0.5525	0.0050	0.0089	1.740(2) ^{ns}
<i>Got-2</i>	0.4919	0.4076	0.0844	0.1715	21.605(2) ^{***}	0.4020	0.3973	0.0047	0.0118	1.439(2) ^{ns}
<i>Got-3</i>	0.5200	0.5074	0.0126	0.0242	4.321(2) ^{ns}	0.3651	0.3425	0.0226	0.0620	9.866(2) ^{**}
<i>Pgd-1</i>	0.6269	0.6089	0.0180	0.0287	8.447(2) [*]	0.5169	0.5068	0.0101	0.0195	5.271(2) ^{ns}
<i>Lap-3</i>	0.4874	0.3431	0.1443	0.2961	37.306(2) ^{***}					
<i>Tpi-2</i>	0.4819	0.4776	0.0043	0.0088	1.112(2) ^{ns}	0.6202	0.6201	0.0001	0.0001	0.066(2) ^{ns}
<i>Tpi-3</i>	0.5668	0.5600	0.0068	0.0119	2.294(2) ^{ns}					
<i>Fe-2</i>	0.4717	0.4496	0.0221	0.0468	5.899(2) ^{ns}					
<i>Fe-3</i>	0.6159	0.5565	0.0594	0.0964	32.992(2) ^{***}					
Mean	0.4726	0.4433	0.0292	0.0601		0.4525	0.4331	0.0195	0.0451	

with that for *H. tsushimensis* (0.453) (Table 4). The proportions of allozyme diversity among populations (G_{ST}) of *H. venusta* and *H. tsushimensis* were 0.060 and 0.045, indicating low levels of genetic differentiation among populations. Only six of 16 polymorphic loci for *H. venusta* and three of 11 polymorphic loci for *H. tsushimensis* revealed significant differences in allele frequencies among populations (Table 4). Average genetic identities among the *H. venusta* and *H. tsushimensis* populations were 0.934 and 0.974. Mean genetic identities among the two species was 0.764.

Discussion

The review of allozyme diversity in plant populations by Hamrick and Godt (1989) provides information for interpreting the allozyme data presented here. *Hosta venusta* and *H. tsushimensis* exhibit considerably more genetic variation than that for plant species with similar life history traits (e.g., long-

lived herbaceous species with a predominantly animal-outcrossing mode of reproduction and wind-dispersed seeds, and endemic geographic range). Mean percent polymorphic loci within populations of species with these life history traits is 35%, mean number of alleles per locus is 1.53, and 1.15 mean effective number of alleles per locus. In addition, the mean values of genetic diversity (H_e) within populations of *H. venusta* and *H. tsushimensis* (0.296 and 0.215) were substantially higher than those for species with similar life history traits (0.113). The similar results were observed in *H. yingeri* S. B. Jones. Although this species is only restricted to remote islands off the southwestern coast of Korea such as Taehuksan, Sohuksan, and Hong Islands (Chung and Kim 1991), it maintains higher levels of genetic variation within populations ($H_e = 0.250$; Chung and Chung 1994).

In general, geographic range has been shown to be significantly associated with the levels of variation maintained within populations (Hamrick, Linhart and

Mitton 1979, Hamrick and Godt 1989). Widely distributed plant species tend to maintain more variation than more narrowly distributed species. Contrary to the expectation, *H. venusta* and *H. tsushimensis*, species with a restricted geographic range, have higher levels of genetic variation than mean estimates of genetic diversity within populations for more widespread their congeners such as *H. capitata* (Koidz.) Nakai ($He = 0.153$; Chung 1994a) and *H. clausa* Nakai ($He = 0.082$; Chung 1994b). Species with small, isolated populations might be expected to have less genetic diversity than species with large and contiguous populations (Chung and Kang 1994). Most populations of *H. capitata* and *H. clausa* are small and isolated (Chung et al. 1991a), which seems to be one of the major factors contributing to lower levels of genetic variation within the species.

The relatively high levels of genetic variation found in *H. venusta* and *H. tsushimensis* is consistent with several aspects of their biology. *Hosta venusta* and *H. tsushimensis* appear to be predominantly outcrossing because flowers had spatial separation (herkogamy) of mature anthers and stigma impeding self-pollination. *Hosta venusta* and *H. tsushimensis* are relatively common in Cheju and Tsushima Islands (Fujita 1976; Chung pers. obs.). It has been observed that seeds are dispersed by wind and pollinator visitation areas are found to be large, which consistent with the results that, on the whole, allele frequencies in populations of both species were close to Hardy-Weinberg expectations. Species that evolve from hybridization or through polyploidy should have enhanced levels of genetic variation. The chromosome number is relatively high ($2n = 60$; Chung et al. 1991a), *H. venusta* and *H. tsushimensis* were suggested as a polyploid origin (Chung et al. 1991a). The combination of these factors may contribute to maintain high levels of genetic variation within the two species. It is of interest to note that populations of *H. minor* (Baker) Nakai, a Korean endemic species oc-

curred in the eastern and southern Korean Peninsula, maintain similar levels of genetic variation with those of *H. venusta* and *H. tsushimensis* ($He = 0.230$; Chung 1994c). This may support that the three species are of similar phylogenetic histories (Chung 1991, Chung et al. 1991b), or ecological and life history traits (Chung 1994c).

Genetic differentiation among populations is principally a function of gene flow among populations via pollen and seeds dispersal (Ellstrand and Marshall 1985). Of the total variation in *H. venusta* ($G_{ST} = 0.060$) and *H. tsushimensis* ($G_{ST} = 0.045$), 6% and slightly less than 5% are due to difference among populations. This is supported by relatively homogenous allele frequencies between populations for the two species. Only six and three out of 16 and 11 polymorphic loci for *H. venusta* and *H. tsushimensis*, respectively, were found to be significantly different in allele frequencies between populations. The levels of divergence found in *H. venusta* and *H. tsushimensis* are substantially lower than the means for species with endemic geographic range ($G_{ST} = 0.248$), outcrossing-animal breeding system ($G_{ST} = 0.197$), seed dispersal by wind ($G_{ST} = 0.143$), and long-lived herbaceous perennials ($G_{ST} = 0.213$) (Hamrick and Godt 1989). However, too few populations were surveyed for each species to draw any conclusions about estimating gene flow by indirect method.

In summary, it is highly probable that the higher levels of variability and lower degrees of differentiation found within and between populations of *H. venusta* and *H. tsushimensis* than in species with similar life history traits would suggest that higher effective population sizes are maintained in the species resulting from extensive gene flow via seed dispersal by wind, large population sizes, and high fecundity (Chung and Chung 1994).

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鄭 明基：オトメギボウシとツシマギボウシにおける遺伝的多様性

済州島および対馬にそれぞれ固有のオトメギボウシ *Hosta venusta* とツシマギボウシ *H. tsushimensis* の遺伝的多様度と集団構造を同位酵素多型の分析により調べた。これらは全体として高レベルの遺伝的多様度を保有しているが、集団間分

化の程度は同属で広い分布域をもっているカンザキギボウシ *H. Capitata* や *H. clausa* などよりも小さかった。このことから、調べた2種は大きな有効集団サイズを持っているのではないかと推定される。